

## Nutritional Enrichment of Apple Pomace by Fungal Fermentations

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### ABSTRACT

This study tested effects of optimised fungal fermentation conditions on nutritional enrichments of apple pomace. Three fermentation experiments were conducted by using  $2.5 \times 10^6$  spores of *Pleurotus ostreatus* (*P. ostreatus*),  $2.5 \times 10^6$  spores of *Phanerochaete chrysosporium* (*P. chrysosporium*) and without microorganisms per g substrate, respectively, for 0, 7, 14 and 21 days using a pilot bioreactor. At the end of each incubation period, 3 steril sample were analysed to determine microbial growth, pH and nutrient contents levels. The results indicated significantly increased crude ash and protein contents ( $P < 0.05$ ) as well as decreased crude fiber and reducing sugar contents of apple pomace by both fungal microorganisms ( $P < 0.05$ ). Crude fat content increased by *P. ostreatus* fermentation while *P. chrysosporium* fermentation reduced crude fat content ( $P < 0.05$ ). Tannin content reduced at all fermentation periods by *P. ostreatus* while *P. chrysosporium* fermentation increased tannin content ( $P < 0.05$ ). Pectin increased by both fungal fermentations, but the effect of *P. ostreatus* was greater ( $P < 0.05$ ). It was concluded that fungal fermentations caused to remarkable improvements of apple pomace in nutritional properties, which could of high importance in animal nutrition.

### Research Article

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## Fungal Fermantasyonu ile Elma Posasının Besin Madde İçeriğinin Zenginleştirilmesi

### ÖZET

Bu çalışmada, elma posasının optimum koşullarda fungal fermentasyonuna tabi tutularak besin madde içeriğinin zenginleştirilmesi amaçlanmıştır. Pilot bir biyoreaktör kullanılarak elma posası, mikroorganizma kullanılmadan,  $2.5 \times 10^6$  spor  $g^{-1}$  *Pleurotus ostreatus* ve  $2.5 \times 10^6$  spor  $g^{-1}$  *Phanerochaete chrysosporium* kullanılarak 21 günlük (0, 7, 14 ve 21 günde örnekler alınmıştır) 3 farklı fermantasyona tabi tutulmuştur. İnkübasyon süreleri sonunda mikrobiyal gelişim, pH ve besin madde içeriğinin tespiti için 3 adet steril örnek alınmıştır. Elma posasının her iki fermantasyonda da ham kül ve protein içeriği artarken; ham selüloz ve redükte şeker içeriğinin azaldığı tespit edilmiştir ( $P < 0.05$ ). *P. ostreatus* fermantasyonunun tüm inkübasyon zamanında tanin içeriği azalırken; bunun aksine *P. chrysosporium* fermantasyonunda artmıştır ( $P < 0.05$ ). Pektin içeriği ise her iki mikroorganizmanın fermantasyonunda da artmış, ancak *P. ostreatus* fermantasyonunda artış oranı daha fazla olmuştur ( $P < 0.05$ ). Elma posasının fungal fermantasyonu ile besin madde içeriği iyileştirilmiştir. Bu araştırma sonucunda fermente elma posasının hayvan beslemede önemli bir yem kaynağı olarak kullanımı ortaya çıkmıştır.

### Araştırma Makalesi

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### INTRODUCTION

There is an increasing trend in adding value to or

utilizing from ligno-cellulotic by-products for the production of several functional bio-molecules

including enzymes, antioxidant and organic acids by the means of microbial fermentation. Apple pomace is a ligno-cellulotic by-product of fruit juice manufacturing sector, which annually processes approximately 20-40% of a total 83 million tonnes apple produced in 2017 (FAO 2018). Apple pomace composed of peel, nut and pulp, which contained about 36% insoluble and 15% soluble fractional fibres on a dried matter basis (Sudha et al., 2007).

Apple pomace of food and feed materials are cheap and rich source of carbohydrate, pectin, crude fibre and minerals in nutritional content (Kara and Doymaz, 2015). It can also provide an economic contribution to livestock production as a means of recycling as an industrial by-product that can cause environmental pollution (Waldbauer et al., 2017; Ulger et al., 2018; Ricci et al., 2019; Matejova et al., 2019). On the other hand, apple pomace can be added to the rations in ruminant animals, in fresh, dried or silage, in poultry as to dried form in amounts that will not have negative effect on animal health (Sudha et al., 2007; Ulger et al., 2018; Yasar and Tosun, 2019a). However, apple pomace has a high-water content of having difficulty for storage, has been seasonally produced (Sharma et al., 2017; Yasar and Tosun, 2019a). These can limit the use of apple pomace in livestock as a source of feed. On the other hand, some antinutritional factors are present in the apple pomace (Ajila et al., 2015). Although rumen fermentation can overcome the antinutritional factors of apple pomace. But the performance of monogastric young animals could be lowered by the use of apple pomace. Therefore, the antinutritional contents of apple pomace should be lowered and its nutritional content must be increased before fed to monogastric animals. One of the treatments to improve nutritional qualities of apple pomace is solid state fermentation (Joshi and Devender, 2006; Mukherjee et al., 2016).

Fungal fermentations of agricultural waste products in solid state fermentation (SSF) resulted in remarkable nutritional and chemical benefits in respect to adding value to these products (Kurt and Buyukalaca, 2010). Several fungal microorganisms were successfully used in various conditions of SSF using apple pomace for the enrichment of nutrients (Villas-Boas et al., 2003; Albuquerque et al., 2006; Vendruscolo et al., 2008; Ajila et al., 2015; Madrera et al., 2017), for improvements of antioxidant enzyme activities (Zheng and Shetty, 2000; Joshi and Devender, 2006; Ajila et al., 2011) and aromatic compounds (Ricci et al., 2019).

Apple pomace fermented with *Candida utilis* has increased crude protein (%100) and mineral (%60) content, while significant reductions in free sugar (%97) content were reported (Villas-Boas et al., 2003). When fermented with *S. cerevisiae* the nitrogen and fat content of apple pomace have been reported to be increased (Joshi and Devender, 2006). Apple pomace

fermented with 3 yeast strains (*S. cerevisiae*, ref: 32; *S. bayanus*, ref: C6; and *H. uvarum*, ref: 62) for 7 days has increased crude protein, fat and dietary fibre content, but depleted the sugars content (Madrera et al., 2017). As a result of fermentation with *Saccharomyces cerevisiae* AXAZ-1 and *Kluyveromyces marxianus* IMB3, some industrial by-products had significantly increased crude fat and protein contents, and *Kluyveromyces marxianus* was found an ideal microorganism for increasing crude protein and fat content (Aggelopoulos et al., 2014).

According to the results, when the apple pomace was fermented with white-rot fungal there was an increase in the content of free sugar, a decrease in the content of crude fibre, ADF and NDF contents (Zhong-Tao et al., 2009; Yasar and Tosun, 2018a). On the other hand, the study conducted by Yasar and Tosun (2019b) showed that the crude ash, ether extract and starch contents of apple pomace was increased by the fermentation of apple pomace with *K. marxianus*, whereas the crude protein, total reducing sugar, crude fibre, ADF, NDF and lignin contents were reduced. Furthermore, apple pomace has been used to produce organic acid and lactic acid (Dhillon et al., 2011; Dhillon et al., 2012; Yasar and Tosun, 2019b). There were sporadic effects of SSF on the changes in the tannin and pectin by microbial SSF (Dhillon et al., 2012; Yasar and Tosun, 2019b).

In this study, the optimum conditions of pH, fermentation periods, stirring rate and moisture content of substrate selected from the above studies were fixed, optimised and controlled by a modern bioreactor and used to ferment apple pomace by two fungal microorganisms, *Pleurotus ostreatus* (Jacquin: Fries) Kummer. teleomorph (ATCC® 34673™) and *Phanerochaete chrysosporium* Burdsall. teleomorph (ATCC® 24725™). The objective of this study was to determine the effects of optimised fermentation conditions selected for two fungal microorganisms on the nutrient fortification of apple pomace, a waste-product of apple juice production.

## MATERIALS and METHODS

Apple pomace were purchased from a local provider dried and ground to pass a sieve with 3 mm and supplemented with additional nutrients (Table 1) and were further autoclaved at 120 °C for 15 min. Two fungal microorganisms, *Pleurotus ostreatus* (*P. ostreatus*) and *Phanerochaete chrysosporium* (*P. chrysosporium*) were obtained from DSM were cultivated according to the supplier instruction to collect sufficient amount of spores for inoculation. Optimum fermentation conditions selected from the literature (Ajila et al., 2011; Pathania et al., 2017; Yasar and Tosun, 2019b) were fixed in the study (see Table 1) and optimized by using a laboratory bioreactor of 2-3 L working capacity, LabforEtOH 5 (Infors Ltd.,

Switzerland), ideally suitable for SSF. A blank fermentation experiment was conducted with no fungal inoculation. The bioreactor automatically well optimized the fixed pH values with peristaltic pumps

using buffer solutions of 0.1 M sodium acetate (pH=1.5) and 0.1 M sodium bicarbonate (pH=9.75) (Pfanckoch, 2001).

Table 1. Experimental design with optimised fermentation parameters fixed throughout the fermentation period  
*Çizelge 1. Fermantasyon süresi boyunca optimize edilmiş fermantasyon parametreleriyle deneme deseni*

Experiments*	Spore g <sup>-1</sup>	pH	Moisture, % (w w <sup>-1</sup> )	Temperature (°C)	Stirring (RPM)	Aeration (L min <sup>-1</sup> )	Days
	<i>Spor</i> g <sup>-1</sup>		<i>Nem</i> , % (g g <sup>-1</sup> )	<i>Sıcaklık</i> (°C)	<i>Karıştırma</i> (RPM)	<i>Havalandırma</i> (L dk <sup>-1</sup> )	<i>Gün</i>
I ( <i>P.ostreatus</i> )	2.5 x 10 <sup>6</sup>	3.0-3.5	65	24-28	10 rpm for 2 min at every 12 h	0.25	0, 7, 14, 21
II ( <i>P.chrysosporium</i> )	2.5 x 10 <sup>6</sup>	5.5-6.0	80	34-38			
III** (None)	0	5.5-6.0	80	24-28			

\*Apple pomace in experiment I, II and III was supplemented by the nutrients as follows: 20 g (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 10 g NH<sub>4</sub>Cl and 10 g CH<sub>4</sub>N<sub>2</sub>O. \*\*Blank fermentation, a non-pH optimised experiment (initial pH of 5.5 did not change throughout the fermentation period) under sterile fermentation conditions, the same as in experiments I and II.

At each sampling time, 3 sterile independent samples were taken from each of fermentation experiments (Table 1) were analysed in triplicates for the determinations of nutritional and antinutritional factors parameters, resulting in 9 replicates per treatment, and the data was analysed according to a general linear model of variance analysis, where the differences between the treatments were separated at 0.05 significance level using a SPSS 2013 software (IBM SPSS Statistics 22.0 for Windows). Fungal growth was determined by the method of TS ISO 21527-2: 2008 and nutrient contents by the methods specifically expressed in AOAC (2005). Metabolic energy values were calculated by Janssen (1989). Pectin Wang and Zhang (1999) and tannin Chemesova and Chizhikov (2004) were spectrophotometrically

analysed. All chemical analysis results were expressed as percentage of dry matter.

## RESULTS and DISCUSSION

The use of a modern bioreactor to optimise all the fermentation conditions was very successful. It was observed that the stirring, aeration and pH were excellently managed without any fluctuations from the fixed values.

As compared to experiment III, where no microbial development was seen, the fungal growth of *P. ostreatus* and *P. chrysosporium* in experiment I and II reached to a maximum level by 4 log increase at 14 and by 2 log increase at 7 days of fermentation, respectively (Table 2).

Table 2. Fungal growth rate and pH of fermenting substrate at 0, 7, 14 and 21 days of fermentation

*Çizelge 2. 0, 7, 14 ve 21 günlük fermantasyonda fungal gelişim oranı ve fermente substratın pH değeri*

Days	Experiments I ( <i>P. chrysosporium</i> ) <i>Deneme I</i>		Experiments II ( <i>P. ostreatus</i> ) <i>Deneme II</i>		Experiments III (None) <i>Deneme III</i>	
	cfu g <sup>-1</sup>	pH	cfu g <sup>-1</sup>	pH	cfu g <sup>-1</sup>	pH
0	3.0x10 <sup>5</sup> ±0.05 <sup>a</sup>	5.50±0.10	7.9 x10 <sup>5</sup> ±0.05 <sup>a</sup>	3.65±0.05	0.0±0.05	5.50±0.05
7	5.9x10 <sup>7</sup> ±0.21 <sup>b</sup>	5.60±0.15	1.9x10 <sup>8</sup> ±0.25 <sup>b</sup>	3.60±0.10	0.0±0.10	5.45±0.10
14	5.7x10 <sup>7</sup> ±0.40 <sup>b</sup>	5.40±0.10	2.0x10 <sup>9</sup> ±0.52 <sup>c</sup>	3.50±0.10	0.0±0.15	5.40±0.10
21	6.4x10 <sup>7</sup> ±0.50 <sup>b</sup>	5.50±0.10	3.0x10 <sup>9</sup> ±0.35 <sup>c</sup>	3.50±0.10	0.0±0.15	5.45±0.10

<sup>a, b, c</sup> Different letters in the same column show significant differences (P<0.05). cfu: Colony-forming unit.

Table 3 contained all analytical data of the apple pomace fermented for 0, 7, 14 and 21 days without fungal inoculants (Experiment III). It can be seen that there were no statistically significant changes in any parameters over the periods of fermentation (days). The effects of fungal inoculants on each of parameters were presented in Figure 1, 2 and 3 as percentage decrease or increase from the blank fermentation at each fermentation period; the analysed values of blank samples were set as "0".

The fungal fermentations remarkably utilised from readily available carbohydrates as the microorganisms

of *P. ostreatus* (Figure 1.A and 1.B) and *P. chrysosporium* (Figure 2.A and 2.B) significantly (P<0.05) consumed total reducing sugar of apple pomace at all fermentation periods, in comparison with blank fermentation experiment (Figure 1 and 2). Overall, the fermentations with *P. ostreatus* (Figure 1.A) and *P. chrysosporium* (Figure 2.A) significantly (P<0.05) increased crude ash and crude protein of apple pomace, expect there was a significant decrease of ash content of apple pomace in 21 days of *P. ostreatus* fermentation.

Table 3. Chemical, composition of apple pomace fermented for 0, 7, 14 and 21 days without fungal inoculants (Experiment III)

Çizelge 3. Mikroorganizma kullanılmadan 0, 7, 14 ve 21 gün fermente edilen elma posasının besin madde içeriği (Deneme III)

Nutrient (DM)	0 d	7 d	14 d	21 d
Crude ash, (%) ( <i>Ham kül</i> , (%))	1.15±0.11**	1.20±0.10	1.10±0.05	1.20±0.70
Crude protein, (%) ( <i>Ham protein</i> , (%))	27.4±0.20	27.8±0.30	28.0±0.40	27.5±0.10
Crude fat, (%) ( <i>Ham yağ</i> , (%))	0.40±0.05	0.36±0.02	0.38±0.02	0.39±0.03
Crude fibre, (%) ( <i>Ham selüloz</i> , (%))	36.10±0.50	35.80±0.30	36.20±0.60	36.00±0.50
ADF, %	44.65±0.40	44.00±1.00	45.15±0.60	44.25±0.30
NDF, %	53.37±0.43	53.05±0.55	52.68±0.98	53.01±0.38
Lignin*	8.65	8.46	8.87	8.63
Total reducing sugar, (%) <i>Toplam indirgenmiş şeker</i> , (%)	19.80±0.20	20.50±0.50	20.0±0.90	20.00±0.70
Metabolic energy, kcal kg <sup>-1</sup> * <i>Metabolik enerji</i> , kcal kg <sup>-1</sup> *	1675	1665	1670	1675
Tannin, (%) ( <i>Tanin</i> , (%))	18.00±0.55	18.97±0.43	18.50±0.20	18.60±0.70
Pectin, (%) ( <i>Pektin</i> , (%))	6.00±0.15	6.30±0.20	5.96±0.15	6.00±0.10

\*These are calculated values. DM, Dry Matter. \*\*P>0.05

Total increase in crude ash and protein was significantly (P<0.05) higher in *P. chrysosporium* (Figure 2.A) than those in *P. ostreatus* (Figure 1.A) fermentation.

However, there was a significant interaction between

the fungal fermentations and crude fat contents. A remarkable increase in the content of crude fat, by about 22 folds (from 0.40% to 9.0%), of apple pomace by *P. ostreatus* fermentation, whereas the fermentation of apple pomace with *P. chrysosporium*

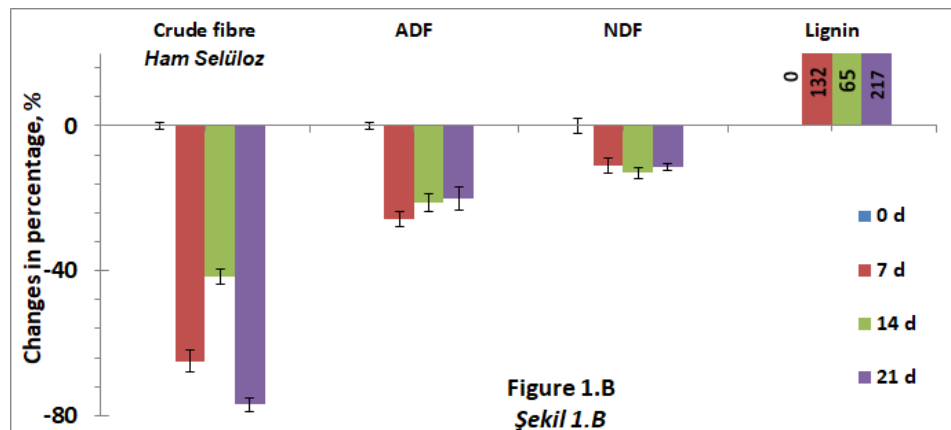
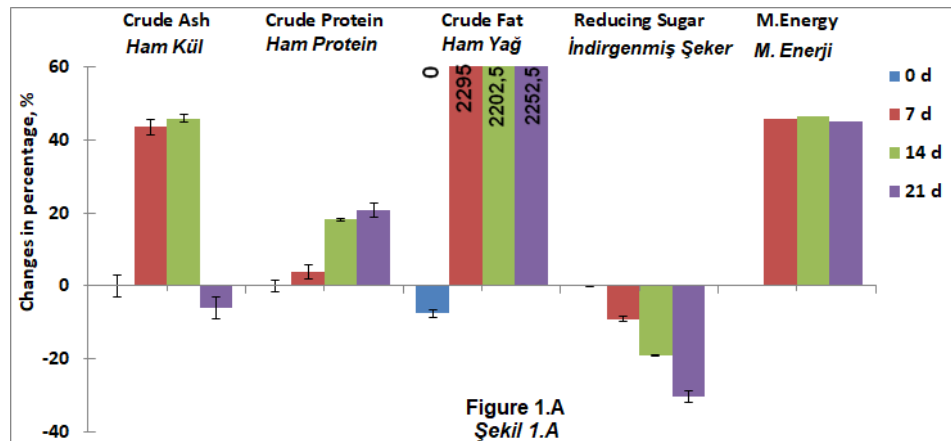


Figure 1. Changes in nutrient contents of apple pomace fermented by *P. ostreatus* (1.A and 1.B)

Şekil 1. Elma posasının *P. ostreatus* ile fermentasyonunda besin madde içeriğindeki değişim (1.A and 1.B)

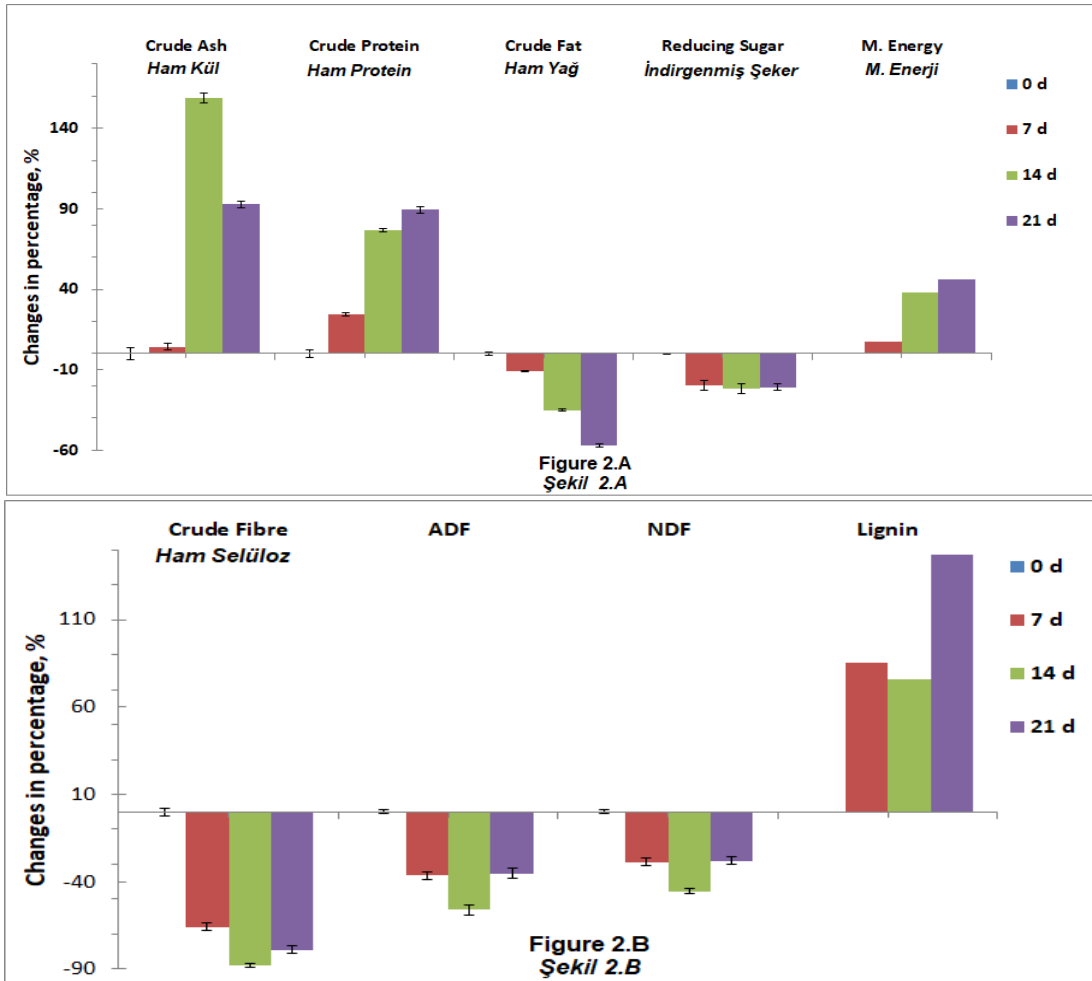


Figure 2. Changes in nutrient contents of apple pomace fermented by *P. chrysosporium* (2.A and 2.B)  
 Şekil 2. Elma posasının *P. chrysosporium* ile fermentasyonunda besin madde içeriğindeki değişim (2.A and 2.B)

significantly reduced the fat contents by up to 50% ( $P < 0.05$ ). On the other hand, the fermentations of both fungal microorganisms significantly reduced the total dietary fibre content of apple pomace up to 80% ( $P < 0.05$ ).

Results in this research were completely agreed with the results of many studies reporting an increase in crude ash and protein content as a result of the fermentation of industrial by-products with various microorganisms including *P. ostreatus* and *P. chrysosporium* (Lateef et al., 2008; Ajila et al., 2015; Madrera et al., 2017; Yasar and Tosun, 2018b). In our study, the rate of increased crude ash and protein content by *P. ostreatus* and *P. chrysosporium* fermentation was comparably higher than these results in the above studies. The reason for the increase in the amount of crude protein in this study is thought to be due to the addition of ammonium sulphate to the medium. Because, Villas-Boas et al. (2003) has been reported that the addition of ammonium sulphate to the fermentation medium has a stimulating effect on fungal and yeast fermentations and increases crude protein production in fermentation. In this study, the reason for the increase

in crude protein content more than other studies is thought to be due to the addition of ammonium sulphate to the medium. Altop et al. (2018) reported that in fungal fermentation microorganisms secrete phytase enzyme and this enzyme breaks down the phosphorus in complex form and as a result, the crude ash content of the substrate increases. In this study, the crude ash content was increased, however, the reason for the increase is not known precisely because no mineral substance analysis was performed. As a result of fermentation of industrial by-products with bacteria, yeast and fungi, it has been determined that the content of crude fat increases by 20-50% (Joshi and Devender, 2006; Madrera et al., 2017; Altop et al., 2018). Similar results were obtained from previous studies of *P. ostreatus* fermentation. In contrast to these studies, the crude fat content of apple pomace was increased by 2252% at the end of fermentation in our study. Unlike the studies in the literature, the crude fat content of apple pomace by *P. chrysosporium* fermentation decreased significantly. This is a first ever scientific outcome from the apple pomace fermentation by *P. chrysosporium*. Therefore, it is highly possible that *P. chrysosporium* needs more fatty

acids for its microbial growth than *P. ostreatus* on apple pomace.

There are many studies reporting decreased crude fibre and its fractions by the solid-state fermentation (Lateef et al., 2008; Yasar et al., 2018; Yasar and Tosun, 2018c; Karakurt et al., 2019). Altop et al. (2018) reported that the starch and sugar content of industrial by-products were reduced as a result of fungal fermentation. In our study, *P. ostreatus* and *P. chrysosporium* microorganism fermentation significantly decreased crude fibre, ADF, NDF and reducing sugar, similar to previous studies. In the fermentation study conducted by Aderemi and Nworgu (2007), it was reported that fungal microorganisms break down structural and non-structural carbohydrates by the enzymes they secrete and as a result of this degradation, crude fibre and its fractions

and sugar contents decrease. Krishna (2005), Aderemi and Nworgu, (2007) and Altop et al. (2018) report that microorganisms secrete enzymes to break down structural and non-structural carbohydrates, thereby breaking down carbohydrates to meet their carbon needs. In this study, it is concluded that microorganisms meet the carbon need for growth and development by breaking down crude fibre, ADF, NDF and reducing sugars and consequently, these contents are reduced.

Fermentation with *P. ostreatus* (Figure 2.A) significantly ( $P<0.05$ ) reduced the tannin contents and meanwhile increased the pectin contents of apple pomace. However, the changes in tannin and pectin contents throughout the fermentation periods were sporadic, not consistent by *P. ostreatus* and *P. chrysosporium* (Figure 2.B).

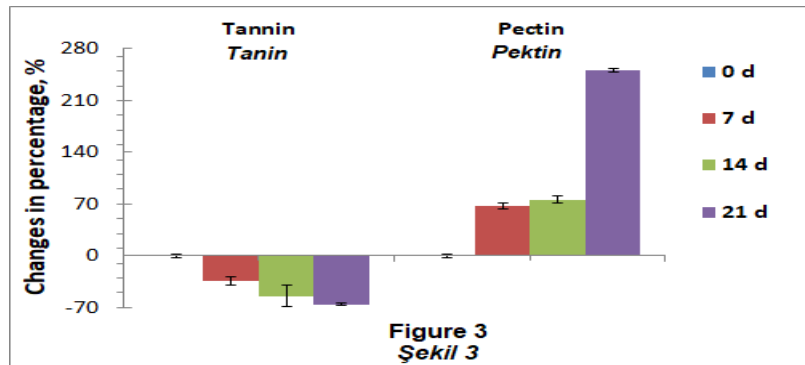


Figure 3. Changes in tannin and pectin contents of apple pomace fermented by *P. ostreatus*  
Şekil 3. Elma posasının *P. ostreatus* ile fermantasyonunda tanin ve pektin içeriklerindeki değişim

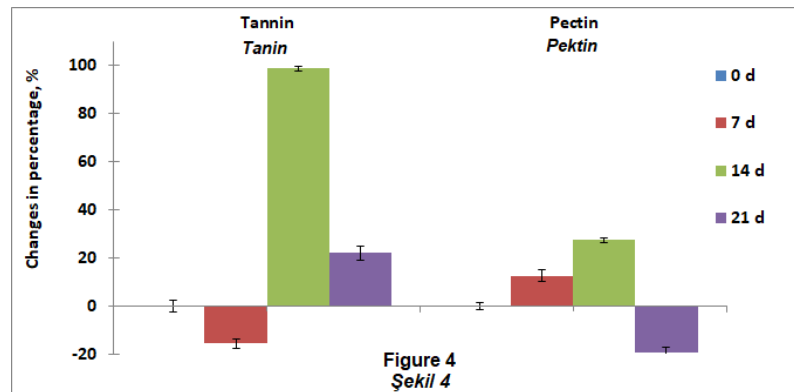


Figure 4. Changes in tannin and pectin contents of apple pomace fermented by *P. chrysosporium*  
Şekil 4. Elma posasının *P. chrysosporium* ile fermantasyonunda tanin ve pektin içeriklerindeki değişim

Degradation of tannin and pectin in apple pomace by fungal microorganisms were also reported earlier (Villas-Boas et al., 2003; Ruiz-Aguilar et al., 2004; Zhong-Tao et al., 2009). In contrary, the magnitude of tannin degradation in our study was larger with the fermentation of *P. ostreatus*, while no degradation of pectin, even led to significant increase in pectin content. Moreover, the tannin content tended to increase by the *P. chrysosporium* fermentation to a significant extend, where the pectin levels had a tendency of decrease towards to the end of

fermentation. Such differences in the pectin content of apple pomace were due to the types of fungal microorganisms differing in the production of pectin esterase enzyme (Joshi et al., 2006; Zhong-Tao et al., 2009; Dhillon et al., 2012; Yasar and Tosun, 2019b).

## CONCLUSION

It can be concluded that the studied fixed fermentation conditions selected from the literature for *P. ostreatus* and *P. chrysosporium* were well suited for the purposes of nutrient fortification of apple pomace in terms of

increased ash and crude protein as well decreased dietary fibre. This result is of significant importance in animal nutrition.

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#### Statement of Conflict of Interest

Authors have declared no conflict of interest.

#### Author's Contributions

The contribution of the authors is equal.

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